Capillary Gel Electrophoresis

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Introduction

Electrophoresis covers a number of bioseparation techniques where charged substances are separated due to differences in migration speed in an electrical field. Since the 1950s, electrophoresis has been routinely used by biochemists to separate, quantify and identify large biopolymers, such as proteins, DNA and complex carbohydrates. In the early days electrophoresis was typically performed in slab or rod-shaped gels, which were necessary to hold the electrophoresis buffer and to minimize the dispersion of the analyte bands by suppressing convection caused by Joule heating and electroosmosis. At that time, electrophoresis was therefore commonly gel electrophoresis.

Electrophoretic separation is based on differences in the charge density (the mass-to-charge ratio) of the analytes. Small, highly charged molecules move faster than large, low charged ones, while uncharged molecules do not move at all. This is a powerful principle, for example for the separation of native proteins and peptides. Other biomolecules such as DNA restriction fragments and carbohydrates are very similar in chemical structure and therefore almost identical in charge densities. For these molecules gel electrophoresis represents a unique opportunity. Gels can be prepared to provide a sieving effect in the relevant mass range and thereby allow separation of charged molecules not according to their charge density but according to their size.

In 1981 Jörgenson and Lukacs published a series of landmark papers, in which they demonstrated both theoretically and practically how electrophoresis could be carried out in free solution as long as capillaries of small inner diameter were used and how the previously unwelcome electroosmotic effect could be used to advantage. The era of (free zone) capillary electrophoresis (CZE) began. Gels were no longer necessary, since the 'wall effect' in capillaries of less than 100 μm i.d. is sufficient to stabilize the flow. Capillary electrophoresis (CE) was seen as a complementary analytical technique to both liquid chromatography and 'conventional' slab/rod gel electrophoresis. In the following decade, research and application of free zone capillary electrophoresis exploded and several variants of the basic technique were established. In 1989 the first commercial CE instrument entered the market.

Capillary gel electrophoresis (CGE) was resurrected in 1983 by Hjerten, albeit for protein separations. It would probably have rested there, since protein CGE subsequently encountered severe problems. Instead it was the human genome project that helped to establish CGE as the important analytical technique it is today. The aim of this project is to determine the sequence of the over 3 billion base pairs that form the human genome. DNA sequencing requires the separation and identification of DNA fragments that differ in length by a single base. The progress of the project is largely determined by the speed of the respective analytical techniques.

The efficiency of CGE is extremely high and up to 3 million plates per metre are routinely reached. Heat dissipation is more effective in thin capillaries compared to conventional gels. As a consequence, much stronger fields can be used in CGE without loss in resolution. This results in significantly reduced analysis times. An example, the single base resolution of oligoadenylates containing from 12 to 60 nucleotides, is shown in **Figure 1**.

Current high throughput instruments use ultrathin capillaries and oligonucleotide primers that are covalently attached to different fluorescent dyes. The restriction digests of all four primers can therefore be analysed in the same sample. A comparison between CGE and conventional gel electrophoresis shows that even though more samples can be analysed in parallel in slab gels, CGE resolves a given sequence at least three times faster. **Table 1** summarizes some additional advantages and disadvantages of CGE over conventional gel electrophoresis.

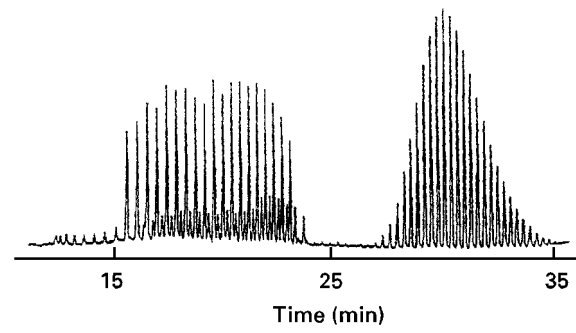

Figure 1 Single base resolution of oligoadenylates containing from 12 to 60 nucleotides by CGE. (Adapted with permission from Baba Y, Matsuura T, Wakamoto K and Tsuhako M (1991) *Journal of Chromatography* 558: 273–284.)

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Table 1 Comparison of capillary and conventional gel electrophoresis

Advantages of CGE
Smaller sample volume
Less buffer, gel and reagent consumption. The latter is especially important, since DNA gel electrophoresis requires toxic and/or mutagenic substances (e.g. ethidium bromide)
Shorter analysis time
Higher resolution
Higher efficiency (over several million plates per metre)
Reliable quantification of the analyte concentration by on column or off column flow through detector (UV absorbency, fluorescence, mass spectrometer, etc.) instead of staining and semiquantitative evaluation in the gel by densitometry
Other than conventional slab gel electrophoresis, CGE can be fully automated from sample injection to separation, detection and data processing steps
Semipreparative applications become much easier, since the molecules pass through and exit the capillary. In conventional gels, the zones are stained within the gel, from which they can only be removed by manual cutting of blotting
Disadvantages of CGE
Restriction in sample size
CGE gels are more difficult to prepare
Capillary coating often necessary to reduce electroosmosis

Gel Filled Columns for Capillary Gel Electrophoresis

Cross-linked chemical gels and physical gels such as agarose are just as popular in CGE as in conventional slab gel electrophoresis. However, since the capillary wall effect exerts its stabilizing influence, solutions of entangled polymers are also used.

Naked fused silica capillaries show electroosmosis above a pH of 3. As a consequence the electrophoresis buffer flows towards the cathode. In capillary gel electrophoresis electroosmosis would lead to extrusion of the gel from the capillary. A possible way to avoid this is to coat the inner silica surface with a neutral polymer. **Table 2** summarizes the more common types of capillary coating. Ideally, the coating should be effective in suppressing electroosmosis as well as the interaction of the analytes with the capillary wall. The preparation should be reproducible and the coating should be stable for a long time and over a wide pH range. Most coatings are attached to the wall by covalent bonds. However, dynamic coatings consisting of ionic, zwitterionic or nonionic molecules that are simply adsorbed to the wall either as monolayer or as bilayer are also used.

Chemical Gels

A general outline for preparing a cross-linked gel column for CGE is given in **Table 3**. Cross-linked polyacrylamide (PAA) gels are prepared by radical copolymerization of acrylamide as monomer and – most frequently – *N*,*N*′-methylenebisacrylamide (BIS) as cross-linker. $(NH_4)_2S_2O_8$ is commonly used as initiator in combination with *N*,*N*,*N*′,*N*′-tetramethyleneethylenediamine (TEMED). Riboflavin (< 5 ppm) has been used as light sensitive initiator but requires the use of UV transparent capillaries. The polymerization of acrylamide can also be started by γ-radiation (^{60}Co source).

The resulting gels have well defined, fairly small average pore sizes of 2 to 8 nm. The %T, %C nomenclature is used for their characterization, with:

$$\%\mathrm{T} = \frac{g_{\mathrm{acrylamide}} + g_{\mathrm{crosslinker}}}{100\ \mathrm{mL\ \ solvent}}$$

corresponding to the monomer concentration:

$$\%\mathrm{C} = \frac{g_{\mathrm{crosslinker}} \times 100}{g_{\mathrm{crosslinker}} + g_{\mathrm{acrylamide}}}$$

corresponding to the crosslinker concentration.

Table 2 Common types of capillary coatings

Coating	*Comments*
Polyacrylamide with Si–O–C bond	Very common, easy to prepare
Polyacrylamide with Si–C bond	Improved hydrolytic stability, difficult to prepare
Polyethylene glycol	Very common
Nonionic surfactants	TWEEN and BRIJ series
LC type stationary phases	C_1, C_8, C_{18}, i.e. weakly, moderately and highly hydrophobic
GC type stationary phases	e.g. DB-17 (50% phenylmethyl silicone)

Table 3 Outline for the preparation of a cross-linked sieving matrix for CGE

Prepare stock solution of monomer, cross-linker, buffer, etc.
Cut/burn detection window if necessary
Pretreat the inner surface of the silica capillary (coating, activation, etc.)
Introduce mixture of monomer (cross-linker)/initiator/catalyst/stabilizing agent (if necessary)
Initiate polymerization
Pre-electrophoresis (to remove impurities and assure constant run conditions and a stable baseline)

Single strand oligonucleotides are typically separated in 2.5%T/3.3%C or 4%T/3.3%C gels. A higher cross-linker concentration gives a tighter sieve and thus higher size selectivity. An increase in the monomer concentration also results in a gel matrix with smaller pores. Lower %T/%C values result in a reduced selectivity but allow coverage of a wider analyte size range. An increased column length can compensate for the loss in resolution, albeit only at the price of increasing the analysis time. A reduction of the cross-linker concentration for a given monomer concentration results in larger pores. A 3%T/0.5%C gel has, for example, been found to give excellent resolution of double-stranded DNA restriction fragments varying from 5 to 12 000 base pairs.

Polyacrylamide is a popular gel matrix because of its electroneutrality. With time, however, it will be hydrolysed to charged polyacrylate, especially at high temperatures (high field strength) and extreme pH values. When the polyacrylate concentration becomes too high, an electroosmotic flow can be observed, which finally results in expulsion of the gel from the capillary. In order to prevent this, the gel may be covalently anchored to the wall coating. This calls for polymerizable (double bonds) groups in the coating. Alternatively, coating and gel formation can be done simultaneously in activated capillaries.

The preparation of gel-filled capillaries is not simple and only four out of five capillaries produced can actually be used. Problems arise in a number of areas, but a major cause of concern is the formation of bubbles during production, transport and use (injection!) of the gel-filled capillaries. Bubbles lead at best to loss in resolution and changing separation patterns and at worst to the total breakdown of the electrical field, especially at high field strengths ($> 300\ \mathrm{V\ cm^{-1}}$).

If the gel and the capillary wall coating are not physically linked, bubble formation is less of a problem, since the wall and the gel can move relative to one another. The reduced stability of the gel under these circumstances is another matter. Whereas capillaries with wall-bound gels can often be used for more than 100 runs, the number of separation that can be performed with the mobile gels tends to be an order of magnitude lower.

Bubble formation during polymerization is largely due to shrinkage of the gel during the process (higher density of the gel than the monomer mixture). **Table 4** summarizes approaches to circumvent this problem. In the case of the laterally aggregated polyacrylamide gels, a hydrophilic polymer such as polyethylene glycol (PEG) is added to the reaction mixture. The more hydrophobic PEG coordinates a large amount of water leaving the growing polyacrylamide strands to form hydrogen bridges mainly among themselves. As a result, thick gel fibres are formed and subsequently stabilized by cross-linking (**Figure 2**). The laterally aggregated gels have larger pores than homogeneous polyacrylamide gels of similar %T/%C. Their sieving behaviour depends also on the concentration and molecular mass of the PEG and should be determined experimentally in each case.

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Physical Gels and Entangled Polymers

In physical gels the network structure is formed by noncovalent interactions such as van der Waals forces and hydrogen bonds rather than chemical

Table 4 Suggestions for the polymerization of bubble-free cross-linked PAA gels

Polymerization at high pressure (400 bar) to equalize the density of the monomer mixture and the final gel
Sequential polymerization starting with the gel formation at one end of the capillary and slowly progressing to the other, for instance by:
• irradiation ($1\ \mathrm{cm\ min^{-1}}$)
• thermal (slow immersion into a heated water bath or slow removal from a cold one)
• isotactic polymerization (the initiator is placed between the leading and the terminating electrolyte and slowly moves through the capillary by isotachophoresis)
Laterally aggregated polyacrylamide gels (formed in the presence of a hydrophilic polymer such as polyethylene glycol)

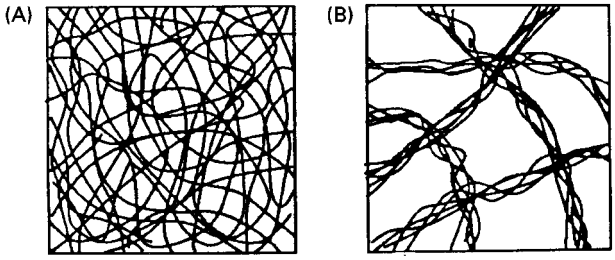

Figure 2 Hypothetical model for 'laterally aggregated' gels. (a) Homogeneous network of cross-linked PAA. (b) Heterogeneous network of PAA in the presence of PEG 2000 (Reproduced with permission from Kuhn R and Hoffstetter-Kuhn S (1993) *Capillary Electrophoresis*: *Principles and Practice*, Springer Verlag.)

cross-linking. If large analyte molecules try to transverse this dynamic structure they also encounter a size-dependent resistance (sieving effect). The type and number of physical gels used in CGE is much more varied than the chemical gels. Mixed physical gels containing, for example, polyacrylamide and cellulose, are also possible. Both solid and liquid sieving matrices are known. Physical gels are often cheaper than chemical gels. They have a more flexible pore structure and can be operated at higher temperatures (50–70°C) and field strength (up to 1000 V cm^{-1}).

Examples of solid physical gels include agarose gels at room temperature and linear polyacrylamide gels with %T > 8 (the %C would be zero in this case). Such high %T polyacrylamide gels also have to be prepared by *in situ* polymerization. The size selectivity is a function of the polymer concentration and can be similar to that of the cross-linked polyacrylamide gels (**Figure 3**). Agarose is a natural polysaccharide network of 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-α-L-galactose. Purified agarose for electrophoresis can be obtained from a number of chemical suppliers. The matrix exhibits a wide range of pore diameters from several hundred nanometres to several micrometres and has high mechanical strength. Agarose is biologically inert and stable between pH 4 and 9. The gel liquefies around 65°C and solidifies again at 35°C. In the molten state it is easily injected into (or removed from) the capillary. Agarose gels can be used below and above the gelling point. Gel filled capillaries (0.3–5% by weight) are stable for a couple of days. No treatment of the capillary walls is necessary. To improve the stability of the gel a small amount of a polyalcohol such as sorbitol may be added.

Entangled polymer solutions are liquid sieving matrices. They are essentially low viscosity gels and can be replaced after each analysis. Hydrolysis or contamination of the network is thus less problematic. Gel formation in entangled polymer solutions depends on the polymer concentration. In dilute solution the polymer molecules are hydrodynamically isolated. For a certain polymer volume fraction Φ^* (overlap threshold) the chains begin to entangle and interact. As a consequence a highly dynamic network is created. Experimentally, Φ^* can be determined by plotting the logarithm of the specific viscosity versus the polymer volume fraction. For $\Phi < \Phi^*$ the curve has a slope of approximately 1.0; for $\Phi > \Phi^*$ the slope increases.

Linear polyacrylamide has to be used at concentrations of at least 6%T to ensure sufficient size discrimination. The solution is then already very viscous and difficult to handle. Other hydrophilic polymers have much lower threshold values. The corresponding solutions are therefore less viscous and can be easily injected and replaced. Among them are various cellulose derivatives (overlap threshold approximately 0.3%), such as methylcellulose, hydroxymethylcellulose, hydroxyethylcellulose and hydroxypropylmethylcellulose. The sieving efficiency depends also on the molecular mass of the cellulose. Molecules weighing more than 900 000 g mol^{-1} make good

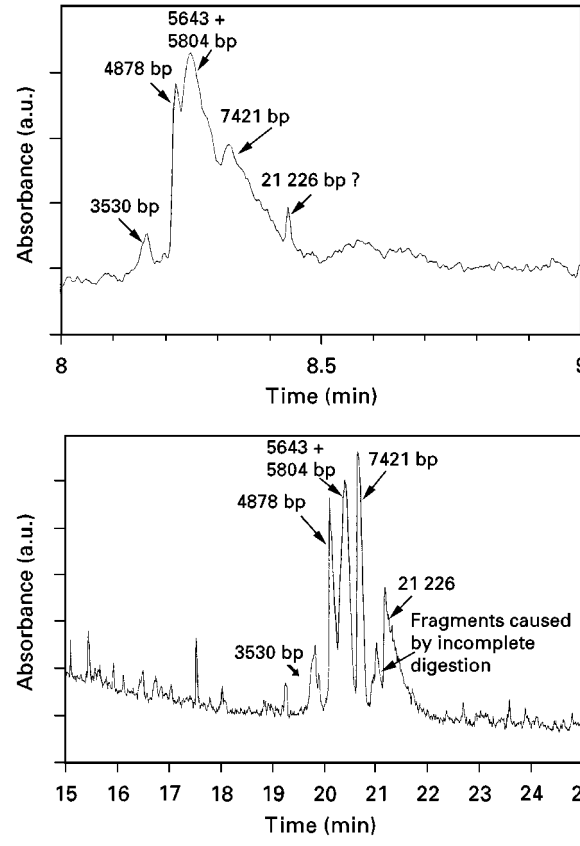

Figure 3 Separation of lambda-DNA restriction fragments (*Eco*RI). Top, 2% polyacrylamide gel; bottom, 4% polyacrylamide gel. (Adapted with permission from Hebenbrock K, Schügerl K and Freitag R (1993) *Electrophoresis* 14: 753–758.)

sieving gels for DNA fragments (polymer concentration 0.5%). For protein separation dextran or polyethylene rather than cellulose derivatives are used to prepare the gels. This has the additional advantage of allowing detection of the proteins at the more sensitive wavelength of 214 nm.

Both physical and chemical gels can be further modified, for example by the incorporation of cyclodextrins or antibodies into the matrix, to allow more specific interactions.

Separation in Capillary Gel Electrophoresis

Two models are used in CGE to describe the separation. The Ogston model considers the gel as a labyrinth of interconnecting pores and channels with an average pore (mesh) size of ζ. The analyte molecules are pictured as rigid spheres with a radius R. Small molecules can pass unhindered through a large fraction of the pores, they therefore move fast but larger molecules are slower. If the pore size of the gel is assumed to be a function of the polymer concentration, the following equations can be used to describe the situation:

$$\mu_g = \mu \exp[-Tb(R + r)^2]$$

and:

$$\mu_g = \mu \exp[-0.25\pi((R + r)\zeta)^2]$$

where μ_g is the analyte mobility in the gel matrix; μ is the analyte mobility in free solution for $E \to 0$; T is the polymer concentration; b is a constant; R is the radius of gyration of the analyte molecule and r is the average radius of the network pores.

The term $b(R + r)^2$ is also called the retardation coefficient, k. To obtain μ, the mobility of the analytes in free solution is extrapolated to a field strength of zero. A plot of μ_g versus T, the Ferguson plot, results in a straight line with a slope of k and an intercept of μ. This plot is used to determine the size separation range of the gel. If the plot is not linear, the assumption that the analytes are rigid spheres with a radius $R < r$ is no longer true.

Biopolymers are flexible molecules rather than rigid spheres and can therefore in reality pass through pores with radii of less then their own radius of gyration. The reptation model considers the analytes as dynamic and essentially chain-like structures that snake and wriggle headfirst through the network in a reptation (hence the name) type of motion. In this model the length of the flexible macromolecule is considered large in comparison to the distance between neighbouring knots in the network. The analyte mobility is proportional to the reciprocal of the chain length or, for nucleic acids, to the base number, N, of the molecule:

$$\mu_g \approx 1/N$$

Larger molecules are again more handicapped than smaller ones. For a flexible macromolecule the transition from the Ogston sieving model to the reptation model takes place when R becomes approximately 1.4 times ζ, depending on the nature (globular proteins, rod-shaped DNA molecules) and the flexibility of their structure. For very large molecules neither model holds true, because the electrical field deforms the molecular structure. For DNA fragments the equation is valid up to approximately 1000 base pairs, depending on the applied field strength. The mobility of larger DNA fragments is actually higher than expected. This leads to a co-migration of larger fragments with smaller ones, especially at high field strength and in high %T gels.

Instrumentation and Methodology

CGE requires no special instrumentation and can be carried out on the same type of instrument as standard capillary electrophoresis. All that is required in addition to the gel filled column is a high voltage power supply and a detector. Most commercial systems are automated to a high degree and also include an autosampler/injection module and a data handling station.

CGE uses continuous electrolyte systems. The separation of the analyte molecules is therefore a kinetic process. In general, all buffer systems for capillary electrophoresis can also be used in CGE. By far the most popular are TRIS–borate buffers (50 or 100 mM TRIS, 50, 100, 250 mM borate, pH 7.6–8.5, up to 5 mM EDTA), especially for the separation of DNA fragments and nucleotides.

While the separation of the native molecules is possible, denatured molecules are sometimes easier to analyse. In this case, denaturing agents, such as high concentrations of urea (5–8 M) are added to the separation buffer, in order to destroy the tertiary and quaternary structure of the molecules. A special case is polyacrylamide gel electrophoresis of proteins in the presence of the surfactant sodium dodecyl sulfate (SDS-PAGE). The protein structure is fully denatured under these conditions including cleavage of the disulfide bonds by a reducing agent such as mercaptoethanol. The remaining polypeptide chains bind SDS in a constant weight ratio (1.4 g SDS per gram of protein) to yield detergent–protein complexes of

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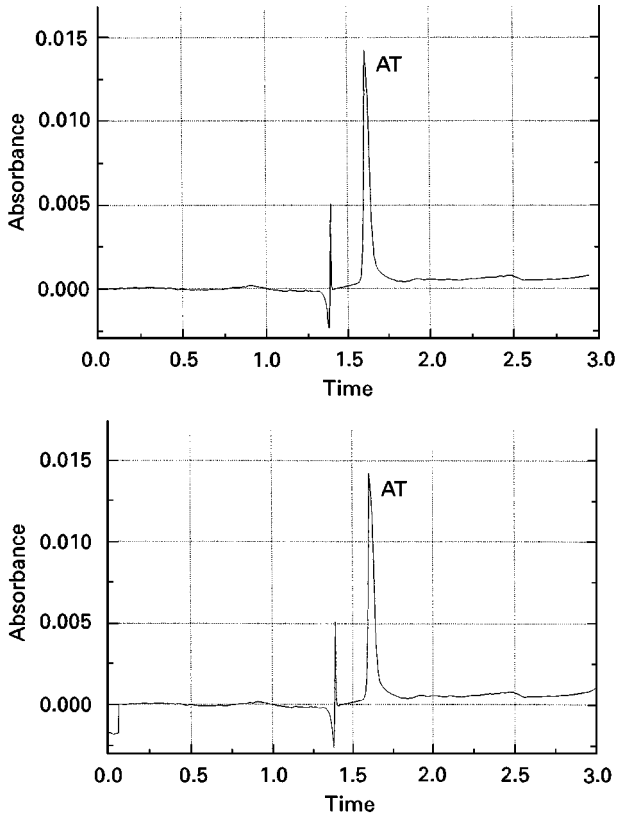

Figure 4 Determination of the molecular mass of recombinant human antithrombin III. Top, calibration using pepsin (34.7 kDa); ovalbumin (45.0 kDa); bovine serum albumin (69.0 kDa); and phosphorylase subunit B (97.4 kDa) as markers. Bottom, recombinant h-AT III. (Reproduced with permission from Reif O-W and Freitag R (1994) *Journal of Chromatography* 680: 383–394.)

constant charge density. SDS-PAGE in slab gels is a standard method for purity control and mass estimation of proteins. SDS-PAGE in capillaries has been used for similar purposes (**Figure 4**). Ethidium bromide is a selective intercalating agent for double stranded DNA. The positively charged molecule can be used to optimize the separation of large DNA restriction fragments in CGE (**Figure 5**). It lowers the charge density and thereby the migration speed of the negatively charged DNA molecules.

Sample Introduction

Two basic types of injection principle are used in capillary electrophoresis. One is injection by pressure (vacuum suction or gravity), i.e. hydrodynamic injection, the other is electrokinetic injection. Pressure injection cannot be used with cross-linked gels, although it may be an option for some of the low viscosity entangled polymer solutions. In most cases it would either not succeed in pushing the sample into the capillary or destroy the gel.

For electrokinetic injection the capillary is placed into the sample vial and the electric field is switched on (typically between 1 and 20 s and 100 and 400 V cm^{-1}). The analyte molecules migrate into the capillary due to electrophoresis. Electrokinetic injection is biased towards small, highly charged molecules which are then over-represented in the introduced sample. In capillary gel electrophoresis the problem is less pronounced, since all analyte molecules are assumed to have identical mass to charge ratios, i.e. differ very little in migration speed.

The surface of the capillary inlet is very important (**Figure 6**). To ensure an even surface, the respective end of the capillary should be cut of with a microtome or snapped off cleanly after scoring with a sapphire cleaver.

Detection

The most common detection principles in CE are optical or column detectors (UV absorbence and (laser induced) fluorescence, LIF). Fluorescence and especially LIF detection allow very low limits of detection (LOD) to be reached. Unfortunately only a few biological molecules show native fluorescence. DNA molecules require pre- or postcapillary derivatization with a fluorescing agent, e.g. a fluorescence-labelled hybridization probe or primer. FITC, JOE, TAMRA, FAM and ROX have been used as labels in DNA sequencing. Proteins have been detected using their native fluorescence at 280 to 340 nm

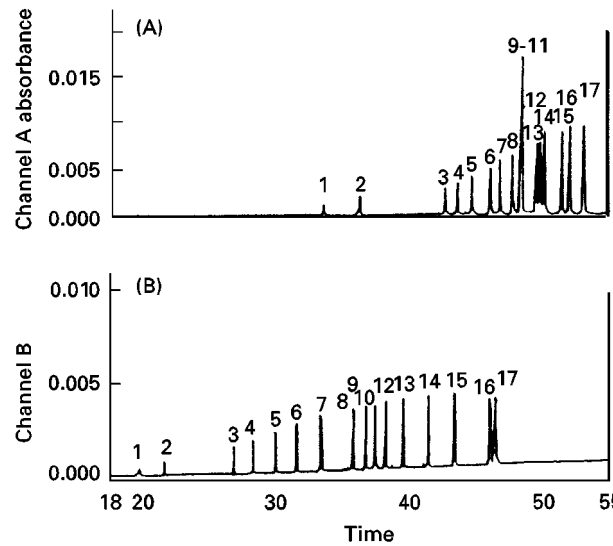

Figure 5 Effect of ethidium bromide addition on the resolution of a restriction fragment digest (pBR 322 by Msp I). Top, no ethidium bromide; bottom, 1 μg mL^{-1} ethidium bromide. (Reproduced with permission from Guttmann A and Cooke N (1991) *Analytical Chemistry* 63: 2038.)

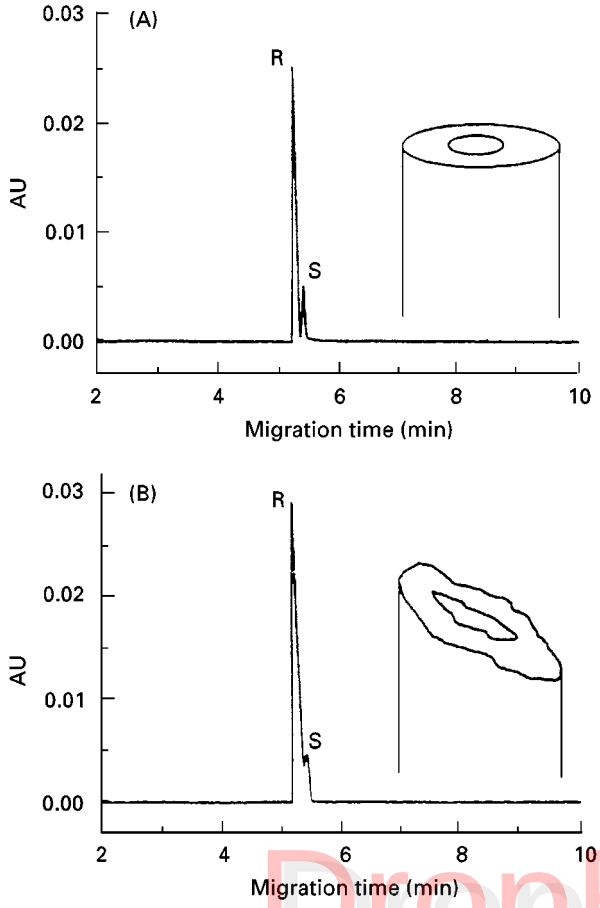

Figure 6 Effect of the physical shape of the inlet of a gel-filled capillary on the resolution of naproxen enantiomers. (Reproduced with permission from Guttmann A and Schwartz HE (1995) *Analytical Chemistry* 67: 2279–2283.)

(tryptophan residues). However, since the quantum yield is often low, labels such as fluorescein isothiocyanate (FITC 494 to 525 nm) are also used (**Figure 7**).

The problem with UV absorbence in CGE is the short optical pathway (inner diameter of the capillary), which leads to low sensitivity. Bubble and z-shaped cells can be used, but the bubble cell makes the capillary fragile, while the z-shaped cell lowers the resolution. In gel-filled capillaries the sensitivity suffers further because of the low UV transparency of most gels. The transparency of a PAA-filled capillary (6%T, 5%C) at 260 nm is 15% lower than that of a water-filled one; even lower if additives like urea or PEG are used. Instead of on-column detection, an off-column detector connected with a sheath flow interface can be used. In addition, several suppliers offer UV-transparent gels either of the cross-linked or the entangled type. The composition of these gels is usually proprietary.

Special Techniques

Varying the magnitude and the direction of the field, so-called pulsed field electrophoresis, can improve the resolution especially for DNA separation, since fragments of up to 10 million base pairs can be analysed. Since the larger molecules are assumed to snake through the gel, they need to orientate themselves in the field before they can advance. If the field is regularly inverted, the advance of the larger molecules is more affected than that of the smaller ones and as a consequence their separation is improved.

Voltage ramping has been shown to improve the separations of mixtures of small and large molecules. First high voltage is used to separate the smaller fragments, afterwards the larger fragments are separated with a gradually or abruptly decreased voltage. Field programming may also aid fraction collection, since the extremely sharp peaks of CGE are difficult to 'catch' unless the analyte migration is reduced by lowering the voltage.

Future Developments

CGE is a rapidly maturing technique and the need for high resolution bioanalytical techniques can be expected to increase rather than decrease in the near future. Up to now application of capillary gel electrophoresis has been dominated by DNA analysis. The potential of the method for protein analysis has not been realized. However, the demands of the modern biopharmaceutical industry for fast and reliable protein characterization techniques may soon change this.

The speed of CGE can be further accelerated by more stable gels (higher field strength) or by using several capillaries in parallel (array). If the sample size could be reduced further, the need for template amplification, e.g. by the polymerase chain reaction (PCR) would be reduced. Detection may be improved by increased use of high molecular weight mass spectrometers (MS). They can be linked to the CE instrument, for example by an electrospray ionization (ESI) interface. As these detectors become more affordable, their use in CGE will increase. Mass spectrometers combine a high sensitivity with being nearly universal detectors. When MS-MS is used more structural information of the analytes become available.

Capillary electrophoresis in many ways is already a microtechnique. However, further miniaturization is possible and the CE on a chip need not be far ahead. Such microchip CE will most likely use gel filled 'capillaries' to realize the maximum number of theoretical plates over the short separation distance.

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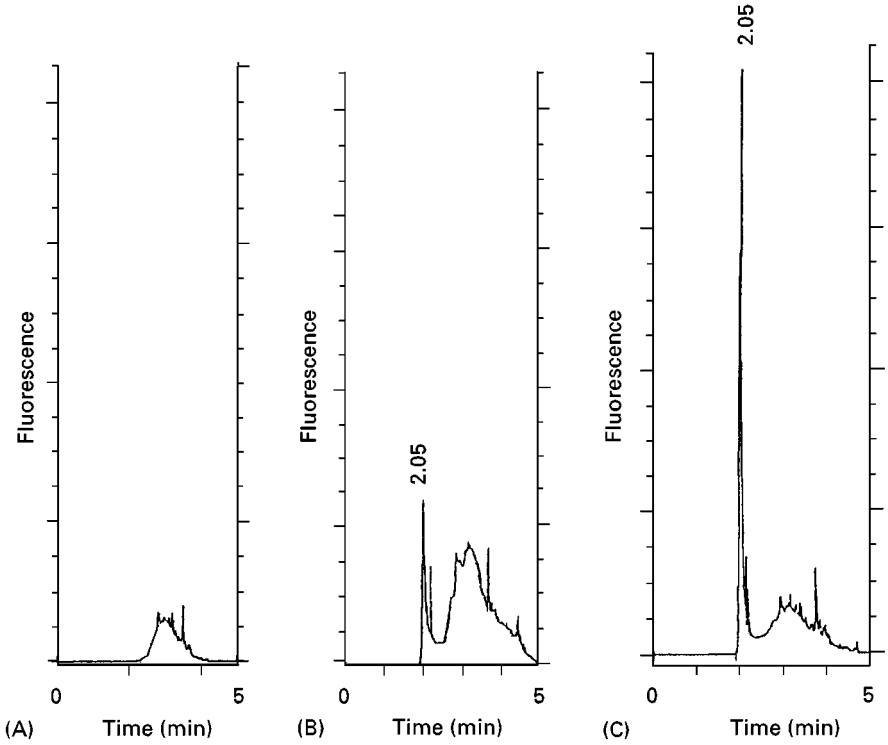

Figure 7 Quantification of human immunoglobulin G (h-IgG) using FITC-labelled Protein G. A, no IgG; B, 250 μg mL^{-1}; C, 1 mg mL^{-1}. (Adapted with permission from Reif O-W, Lausch R, Scheper Th and Freitag R (1994) *Analytical Chemistry* 66: 4027–4033.)

Further Reading

Beale SC (1998) Capillary electrophoresis. *Analytical Chemistry* 70: 279. (This journal prints every two years a review on capillary electrophoresis. The article is written by an expert in the field and covers more or less all developments in CE including CGE of the preceding years.)

Grossmann PD and Colburn JC (eds) (1996) *Capillary Electrophoresis: Theory and Practice*. San Diego: Academic Press.

Grossmann PD and Soane DS (1991) Capillary electrophoresis of DNA in entangled polymer solutions. *Journal of Chromatography* 559: 257. This paper treats the two models for separation in CGE (Ogston and reptation) in more detail.

Jörgenson JW and Lukacs KD (1981) High resolution separation based on electrophoresis and electro-osmosis. *Analytical Chemistry* 53: 1298.

Kuhn R and Hoffstetter-Kuhn S (1993) *Capillary Electrophoresis: Principles and Practice*. New York: Springer Verlag.

Landers JP (ed.) (1994) *Handbook of Capillary Electrophoresis*. Boca Raton: CRC.

Li SFY (1992) *Capillary Electrophoresis*. Amsterdam: Elsevier.

Lunte SM and Radzik DM (1996) *Pharmaceutical and Biomedical Applications of Capillary Electrophoresis*. Oxford: Pergamon Press.

Schwartz H and Guttman A (1995) *Separation of DNA by Capillary Electrophoresis*. Fullerton, CA: Beckmann Primer 607397.

Capillary Isoelectric Focusing

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Isoelectric focusing (IEF) possibly represents the electrokinetic method with the highest resolving power. In IEF, amphoteric compounds are sorted in order of their isoelectric points (p*I*) in a steady-state pH gradient. Good resolution is favoured by both a low diffusion coefficient and a high mobility slope at the p*I*,